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14. ABSTRACT Aneuploidy occurs with high frequency in breast cancer cells, and an aneuploidy increase is positively correlated with the transition from pre-malignant to metastatic cancer. Chromokinesins, a family of chromosome-associated microtubule motors, are potential generators of aneuploidy since they are believed to participate in spindle morphogenesis and chromosome movements during mitosis. Our hypothesis is that inhibition of chromokinesin activity increases the aneuploid frequency in cultured cells. Five different chromokinesins were knocked-down individually or in combination in cultured S2 cells using RNAi. Individual and combined chromokinesin RNAi can result in a statistically significant increase of kinetochore number (ie, aneuploidy). Generally, inhibition of multiple chromokinesins has a stronger affect on aneuploidy than inhibition of individual chromokinesins, but the multiple inhibition affects are not strictly additive. In addition, RNAi of some chromokinesins markedly increased the mitotic index and the frequency of multi-nucleation. Together, these results support the hypothesis that chromokinesin inhibition can lead to aneuploidy.					
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Introduction

Breast cancers are frequently aneuploid (16), and an increase of aneuploidy is positively correlated with the transition from pre-malignant to metastatic cancers (8). Clearly, identifying the cause of this form of genomic instability is important for understanding the genesis of breast cancer and devising possible clinical treatments. In general, aneuploidy results from the improper segregation of chromosomes, resulting in chromosomal loss or non-disjunction, but the details of the events leading to aneuploidy are not well understood.

Mitotic chromosome segregation is a fundamentally important process leading to the equal partitioning of the genomic DNA to two daughter cells. Proper segregation is critical during the rounds of cell division necessary for the normal development of metazoans. Improper segregation usually leads to the loss of daughter cells, but also may initiate a process leading to the development of cancer. It is important to understand the mitotic process, particularly the mechanisms that underlie segregation and the molecules used to construct and vitalize the mitotic apparatus.

The Mitotic Spindle. The mechanics of segregation are performed by an evolutionarily conserved structure, the mitotic spindle. In animal cells, the spindle is a fusiform shaped array of microtubules (MTs) which focus at either end of the spindle at the spindle poles (Figure 1A). Spindle formation begins during prophase of mitosis, when the two closely-positioned spindle poles each nucleate an aster-like cluster of dynamic MTs and begin to move over the nuclear envelope until the poles are positioned at nearly opposite ends of the nucleus. Recent studies of spindle pole separation in animal cells have determined that the activities of specific MT-associated motors are necessary for pole separation (15, 17). Yet other MT-associated motors are believed to maintain pole separation by resisting forces that otherwise cause the poles to collapse together. Dynamic MTs grow from the poles to probe the cytoplasm and, after nuclear envelope break-down, are able to interact with the condensed chromosomes. Some MTs interact with chromosomes at kinetochores (macromolecular complexes associated with chromosome centromeres) while others interact with chromosome arms (Figure 1B). Following interaction with spindle MTs, chromosomes begin to congress -- the process of chromosome movement to a position halfway between the poles. Again, motors are believed to be required for this process. Finally, when all chromosomes are properly positioned and aligned at the spindle's middle (the metaphase plate), the chromatids of each chromosome separate and are segregated to the opposite poles during anaphase. Motors are required for this late stage of mitosis, as well. After segregation of chromatids, the two chromosome masses are re-enveloped in nuclear membranes and daughter cells are separated during cytokinesis.

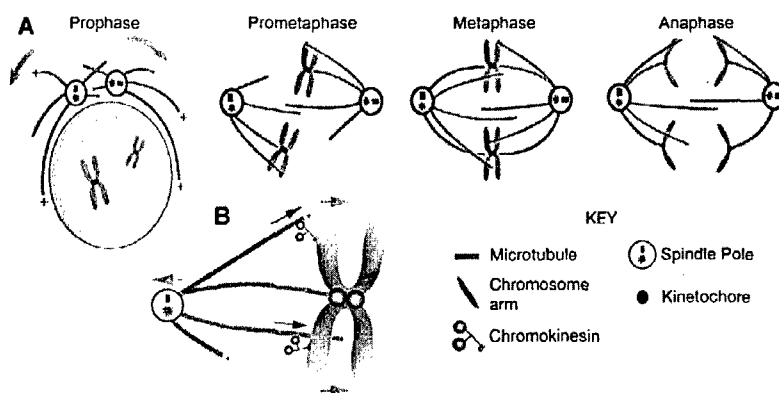


Figure 1. Events of mitosis. A. The four stages of mitosis leading to chromosome segregation. B. Chromokinesins are hypothesized to bind chromosome arms and translocate to the plus-ends of MTs (red arrows). This generates forces (green arrows) that push chromosomes and poles apart.

Chromokinesins. Clearly, motors are central to the construction and function of the spindle (7, 9, 15), but the contributions of all the participating mitotic motors are not known. Every motor has a motor domain that contains the sites for ATP hydrolysis and nucleotide-dependent MT-binding. Outside the motor domains are regions of divergent sequence that may interact with other molecules -- these may be "cargo" molecules / organelles transported by the motor or may be ligands that target the motor to certain intracellular locations.

Chromokinesins are a family of force-producing ATPases that are believed to associate with chromosomes (Figure 1B) and propel them along the microtubule fibers of the mitotic spindle (5). Since chromokinesins participate in chromosome positioning and translocation, then loss of chromokinesin activity should interfere with proper chromosome segregation and, therefore, should increase aneuploid frequency. The behavior of another structure important for chromosome translocation, the kinetochore, is believed to respond to forces applied on chromosomes. Therefore, the force-producing chromokinesins probably influence kinetochore activity, resulting in an integrated system that effectively monitors chromosome position along the spindle and translocates chromosomes to their proper location. If this model is correct, then chromokinesins could be important for chromosome segregation in two ways: by directly propelling chromosomes and by modifying the activity of another chromosome-positioning structure, the kinetochore.

In addition, a chromokinesin has been shown to bind the BRCA2-associated factor, BRAF35, in human cells (6). This finding suggests that proper function (and/or localization) of chromokinesin and BRCA2 could be interdependent.

Table I. Known and predicted *Drosophila* chromokinesins.

Chromokinesin	Mutant <i>Drosophila</i> Phenotypes
Nod	Non-disjunction of achiasmatic chromosomes.
KLP3A	Improper pole separation; abnormal spindles.
KLP31E (putative)	Unknown.
KLP38B	Abnormal spindles; disrupted anaphase.
KLP88A (putative)	Unknown.

Predicted Chromokinesin Function. The polarity of translocation along MTs has been tested for only the human chromokinesin, Kid, and found to be plus-end directed (20). In fact, the translocation polarity for all chromokinesins is predicted to be plus-end directed, since all tested KLPs with amino-terminal-positioned motor domains are plus-end directed and since chromokinesins' motor domains are located within the amino-terminal regions. Therefore, chromokinesins are predicted to transport their cargo towards the plus-ends of MTs. In a mitotic spindle, this would result in the movement of chromosome arms away from the spindle poles and towards the middle region (the metaphase plate) of the spindle, where the MTs emanating from both poles terminate at their plus-ends (Figure 1B). This plateward motion probably accounts for the "polar ejection force" that drives chromosomes to the plus-ends of spindle MTs (10, 11). Another predicted consequence of this activity is that spindle poles should experience a force pushing them away from the metaphase plate; unless this force is counterbalanced, the

spindle could be lengthened. This rationale predicts that chromokinesins could participate in spindle extension and chromosome congression during prometaphase, and chromosome alignment during metaphase. Each of these events is important for successful segregation, and so proper chromosome transmission to daughter cells probably requires chromokinesin activity (19).

The Model System: In eukaryotes, chromosome segregation to daughter cells is performed by the mitotic spindle, an evolutionarily conserved macro-structure whose molecular components generally have been found, to date, to be likewise conserved. Therefore, we chose to perform our studies in *Drosophila* Schneider S2 cells because:

- (1) *Drosophila* chromokinesins display homology with higher eukaryote chromokinesins.
- (2) S2 cells cultured on concanavalin A-coated substrates spread extensively, even during mitosis, allowing excellent microscopic visualization of the spindle apparatus (13, 14).
- (3) S2 cells are easily amenable to dsRNA interference, so protein expression can be selectively inhibited (2, 12, 13).
- (4) An antibody to the kinetochore-specific protein, Cid, is available from another lab. This antibody works in S2 cells and can be used to immunostain kinetochores (Figure 2).
- (5) In addition, S2 cells can be stably transfected with fluorophore-tagged proteins, allowing important structures (eg, spindle MTs, chromosome histones) to be imaged and recorded in live cells.



Figure 2. *Drosophila* S2 cells immunostained to localize the kinetochore-specific protein, Cid. *Drosophila* have 4 chromosomes, so untreated diploid cells are expected to have 8 or 16 kinetochores, depending on cell cycle stage. Following a week-long RNAi treatment, cells are fixed, immunostained, optically sectioned and recorded using a confocal microscope, and then the kinetochores of each cell are counted while stepping through the z-stack.

Relevance: Two rationales highlight the possible connection of chromokinesins to breast cancer: First, since chromokinesin interacts *in vivo* with a BRCA2-binding protein, the regulation of this motor's chromosome-translocating activity may be linked to the BRCA2 system. Second, recent studies have established aneuploidy as a common feature of breast cancers, particularly of progressed cases. The proposed research will determine if aberrations of chromokinesin activity can generate aneuploidy and, therefore, can be a root cause of some cancers. To date, this notion has not been tested. Also, by identifying activity-modifying factors, potential therapeutic agents can be explored.

Body

Hypothesis: The overall goal of the research is to test the hypothesis that abnormalities in chromokinesin activity increase the aneuploid frequency. If chromokinesins are needed for genomic stability, then disrupting chromokinesin activity could generate daughter cells with increased likelihood to become tumorigenic because of their aneuploid state.

Identifying and Cloning Chromokinesins: Some *Drosophila* chromokinesins (eg, Nod, KLP3A) have been previously described in the literature. In order to identify other, putative

chromokinesins, a list of potential kinesin motors was analyzed for those that could function as chromokinesins. Members of the kinesin motor superfamily can be identified by the presence of the canonical kinesin motor sequence. The *Drosophila* genome has been searched to identify all potential kinesin superfamily members (4); from this list, we have selected those that, to date, have not been characterized. To predict which kinesin motors could function as chromokinesins, we analyzed the uncharacterized kinesins' sequences for the presence of nucleic acid binding motifs. Two putative chromokinesins, KLP31E and KLP88A, were identified with this procedure.

Four full-length chromokinesins (Nod, KLP3A, KLP31E, and KLP38B) have been cloned from S2 cell RNA using standard RT-PCR techniques. Several attempts have been made to clone KLP88A, a putative chromokinesin, but the resulting clones invariably contained deletions or duplications; the cause of the sequence corruption is unknown. Currently, we are attempting to clone full-length KLP88A by ligation of cloned KLP88A fragments.

Chromosome Binding: By definition, chromokinesins bind to chromosome arms at some point during mitosis. Therefore, demonstration of a bona fide chromokinesin includes the observation of chromosome arm / chromokinesin binding, either by co-purification or co-localization following immunostaining. Chromosome binding has been previously reported for Nod and KLP3A, but not for KLP31E, KLP38B, or KLP88A. Therefore, an attempt was made to generate rabbit polyclonal antibodies against the chromokinesins, KLP31E and KLP38B. (Because KLP88A has not been successfully cloned, no antibodies have been generated for this motor.)

Constructs of the KLP31E and KLP38B non-motor regions (which display little sequence similarity with other *Drosophila* kinesins) fused to maltose binding protein (MBP) or glutathione-S-transferase (GST) were bacterially expressed, affinity purified, and then used as immunogens in rabbits. (Antigen injections and bleeds were performed by Covance, Inc. and Proteintech Group, Inc.)

KLP38B is found on chromosome arms (as well as at spindle poles) at metaphase, but interestingly, no longer co-localizes with chromosomes during anaphase, when the two sets of chromatids are segregating to opposite poles (Figure 3). This finding has implications for the

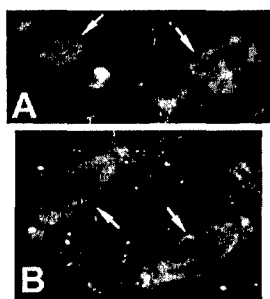


Figure 3. KLP38B immunolocalization in *Drosophila* early embryos. A. During metaphase, KLP38B (blue) localizes to chromosomes (arrows) and spindle poles. Spindle MTs are red. B. At the start of anaphase, KLP38B immunostaining no longer localizes to chromosomes (arrows) but is detectable outside the spindle regions and at centrosomes (white dots at centrosomes result from the overlay of blue [KLP38B] and red [MTs]).

regulation of chromokinesins, since the activities of these motors probably must be terminated during anaphase. Because the expected force produced by chromokinesins would inhibit chromatid movement to poles, a reasonable expectation is that chromokinesin activity diminishes at the start of anaphase in order to allow chromatids to segregate. In *Xenopus* egg extracts, the chromokinesin, Xkid, is proteolyzed at the start of anaphase (1, 3), but its human homolog, Kid, persists on centromeres during anaphase even though localization to

chromosome arms is lost (18). *Drosophila* chromokinesins could be similarly regulated by proteolysis, or alternatively could be inhibited by a reversible modification (e.g., phosphorylation) that displaces chromokinesins from chromosome arms or directly prevents force generation.

S2 cells immunostained with anti-KLP31E serum revealed no specific staining in S2 cells. However, the sera used were early bleeds from two immunized rabbits, raising the possibility that the anti-KLP31E titers in the sera are low. Anti-KLP31E antibodies are being affinity purified from sera of later bleeds in order to re-test the antibodies on S2 cells. In addition, the purified antibodies will be characterized by Western blotting against purified KLP31E tail (the region of KLP31E used for immunization) and S2 cell lysate.

Chromokinesin Inhibition Increases the Mitotic Index: If chromokinesins are required for mitosis, either for assembly/maintenance of the spindle and/or for positioning of the chromosomes within the spindle, then inhibition of these essential functions should trigger the spindle checkpoint and prevent successful completion of mitosis. As a result, cells lacking chromokinesin activity should have a higher mitotic index than controls.

To test this prediction, cultured S2 cells were treated with a week-long regimen of dsRNA applications to induce RNA interference (RNAi) of translation. First, regions of each of the five chromokinesin sequences for RNA synthesis were selected for their uniqueness, minimizing the possibility that the RNAi would have off-target effects. Second, dsRNA was transcribed from DNA templates (either cloned chromokinesins or ESTs that contained the target sequence), and then purified by chloroform/phenol extraction followed by propanol precipitation. The RNA quality was verified by agarose gels and OD₂₆₀/OD₂₈₀ ratios. dsRNA application generally followed the procedure of Clemens et al (2000): cultured S2 cells were exchanged into serum-free medium containing the dsRNA and incubated for 30-60min., and then brought to 10% (final concentration) fetal bovine serum (FBS) in medium by the addition of an equal volume of 20% FBS medium. After four applications of dsRNA on alternate days, the S2 cells were plated on concanavalin-A coated coverslips to promote cell attachment and spreading. Cells were then fixed in 100% methanol, -20°C, and then immunostained as required (eg, with anti-phosphohistone antibody to specifically stain histones of mitotic cells).

Because failure to progress normally through mitosis can induce apoptosis in some cell types (which would lead to the loss of cells blocked in mitosis and thus an under-representation of the RNAi phenotype), we tested if the inclusion of Nc caspase dsRNA in the RNAi treatments would increase the severity of the observed phenotypes. For some treatments, this was found to be true (Figure 4). Therefore, Nc caspase dsRNA was routinely added to all RNAi experiments (including controls).

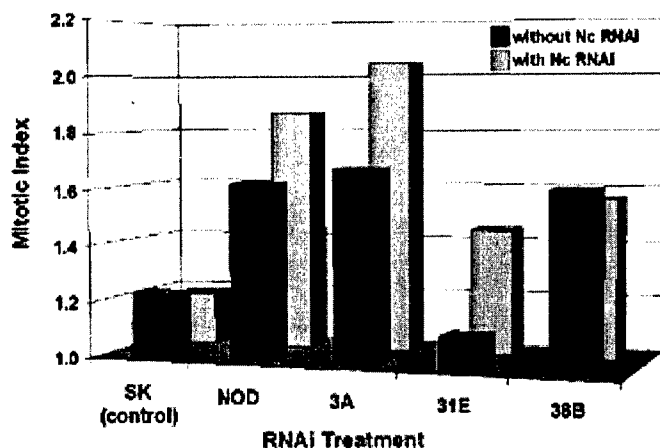


Figure 4. Chromokinesin RNAi increases the mitotic index for all chromokinesins tested. Inclusion of Nc caspase dsRNA (green) generally further increases the mitotic index when combined with chromokinesin RNAi.

Knock-down of chromokinesins was found to increase the mitotic index of S2 cells, indicating that chromokinesins function during mitosis and are required for normal progression throughout mitosis (Figure 4). Since RNAi of the different chromokinesins did not identically affect the mitotic index increase, then the functions of the chromokinesins are probably not identical (though they might be partially redundant).

Multiple knock-down of chromokinesins using combined applications of motor-specific dsRNA to S2 cells generally increased the mitotic index above that obtained by single motor knock-down (Figure 5). Assuming that the week-long RNAi treatment was sufficient to effectively eliminate a motor's function during mitosis, these result of Figure 5 suggests that the different chromokinesins do not have completely redundant functions and that they are not all part of only a single mechanistic pathway. Therefore, the different chromokinesins probably make different functional contributions to mitosis.

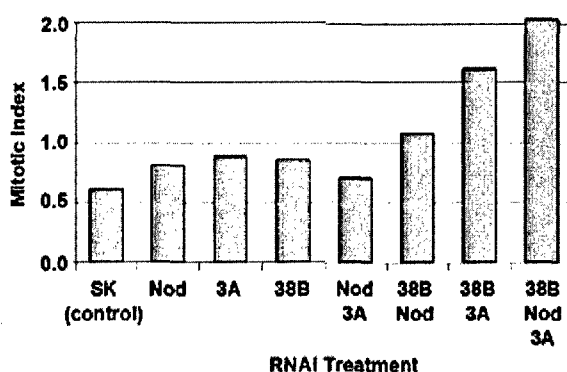


Figure 5. Knock-down of multiple chromokinesins generally increases the mitotic index above that caused by single chromokinesin knock-down.

Chromokinesin Inhibition Increases Aneuploidy: If chromokinesins are required for the correct segregation of chromosomes, then their inhibition should predictably lead to the generation of daughter cells with improper chromosome numbers. To test this prediction, individual chromokinesins were knocked-down by week-long RNAi treatment and then the number of chromosomes per cell was found by counting the number of kinetochores in each cell. Kinetochores were visualized by immunostaining with anti-Cid antibody (Figure 2).

The results of these experiments are plotted in Figure 6 as distribution histograms of the chromatid number per cell. The distributions were analyzed by non-parametric, one-way ANOVA. Individual KLP38B and KLP31E RNAi were found to significantly alter the number of chromatids per cell. All chromokinesin RNAi treatments were found to significantly alter the chromatid-per-cell distribution compared to control (determined by Kolmogorov-Smirnov analysis) (Figure 6).

Aneuploidy resulting from chromokinesin RNAi is manifested as a significant skewing of the distribution towards an increasing chromatid number (Figure 7). For each RNAi treatment, the distribution of chromatid number with three separate categories (<8, 8-16, and >16 chromatids per cell) was analyzed. For the <8 chromatid per cell range, all RNAi treatments had statistically similar distributions, but chromokinesin RNAi caused significant skewing towards a greater number of chromatids per cell in the other two ranges. Therefore, the aneuploidy that arises following chromokinesin RNAi results from too many chromosomes per cell. Aneuploidy in the form of chromosome loss may result in loss of viability, leading to cell death.

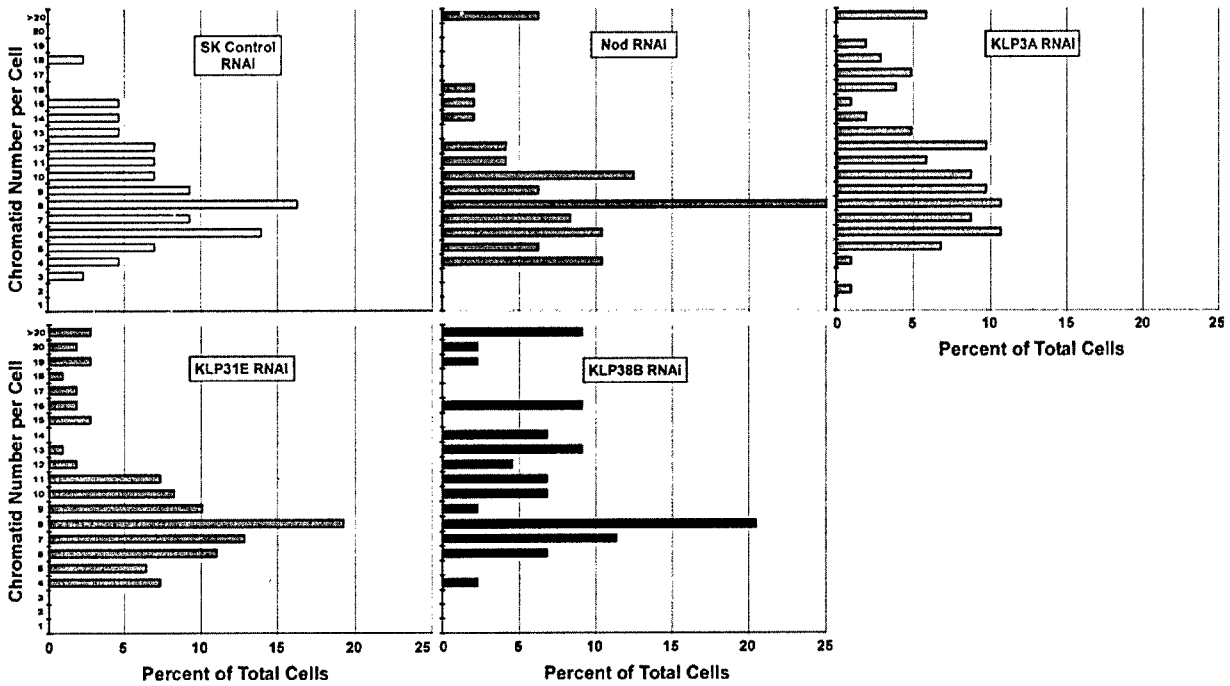


Figure 6. Inhibition of chromokinesins can generate aneuploidy. After RNAi treatment to knock-down chromokinesin targets, the chromosome number per cell was determined in anti-Cid immunostained cells to specifically label kinetochores. Cells were imaged as z-section series by confocal microscopy to facilitate accurate kinetochore counting. KLP31E and KLP38B RNAi treatments significantly alter the median chromatid number compared to the SK negative control. All chromokinesin RNAi treatments alter the distribution of chromatid number compared to SK control.

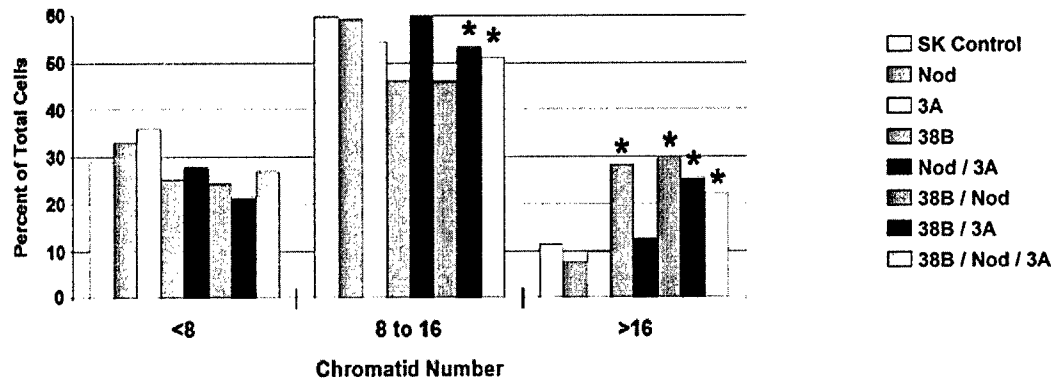


Figure 7. Inhibition of chromokinesins causes aneuploidy by significantly increasing the number of chromatids per cell. Within each range, the data were analyzed for significant differences in chromatid number distribution within that category (Kruskal-Wallis test, $p < 0.05$). Asterisks (*) mark treatments that have chromatid number distributions significantly greater than control (SK). For example, within the 8-16 range, both 38B/3A and 38B/Nod/3A RNAi treatments have a distribution of chromatid number that is significantly skewed higher than control (SK).

Interestingly, these results indicate that loss of the chromokinesin, KLP38B, is particularly prone to generating aneuploidy. Loss of other chromokinesin activities in addition to KLP38B appears to further increase the frequency of aneuploidy.

Live Cell Analysis of Chromokinesin RNAi Treated Reveals Mitotic Abnormalities: The results so far demonstrate the loss of chromokinesin activity can generate aneuploidy, and that the chromokinesins are not equivalent in their capacity to generate aneuploidy following RNAi. Therefore, the mitotic functions of the chromokinesins are not likely to be fully redundant. Determining the actual function of each chromokinesin was initially addressed by recording the assembly and movements of mitotic spindles in live S2 cells (expressing GFP-tubulin to permit visualization of the spindle microtubules) following RNAi. Cells were imaged using a spinning disk confocal microscope in order to minimize photodamage and bleaching. Since many mitotic cells of each treatment must be recorded to obtain a complete collection of representative phenotypes, the results below are preliminary but suggest possible functions for different chromokinesins.

In Figure 8, individual frames were selected from movies of S2 cells (expressing GFP-tubulin) after treatment with RNAi to eliminate the activity of a target chromokinesin. The SK negative control cells proceed normally through mitosis, initially forming a bipolar spindle during prometaphase and eventually segregating chromosomes during anaphase. The Nod RNAi cell forms a bipolar spindle, but the central region of the spindle appears less robust than the control's, suggesting that Nod activity is needed to stabilize the central array of spindle microtubules. This may account for the lack of chromosome segregation apparent in this Nod RNAi treated cell. On the other hand, KLP38B RNAi treatment causes a spindle collapse, which

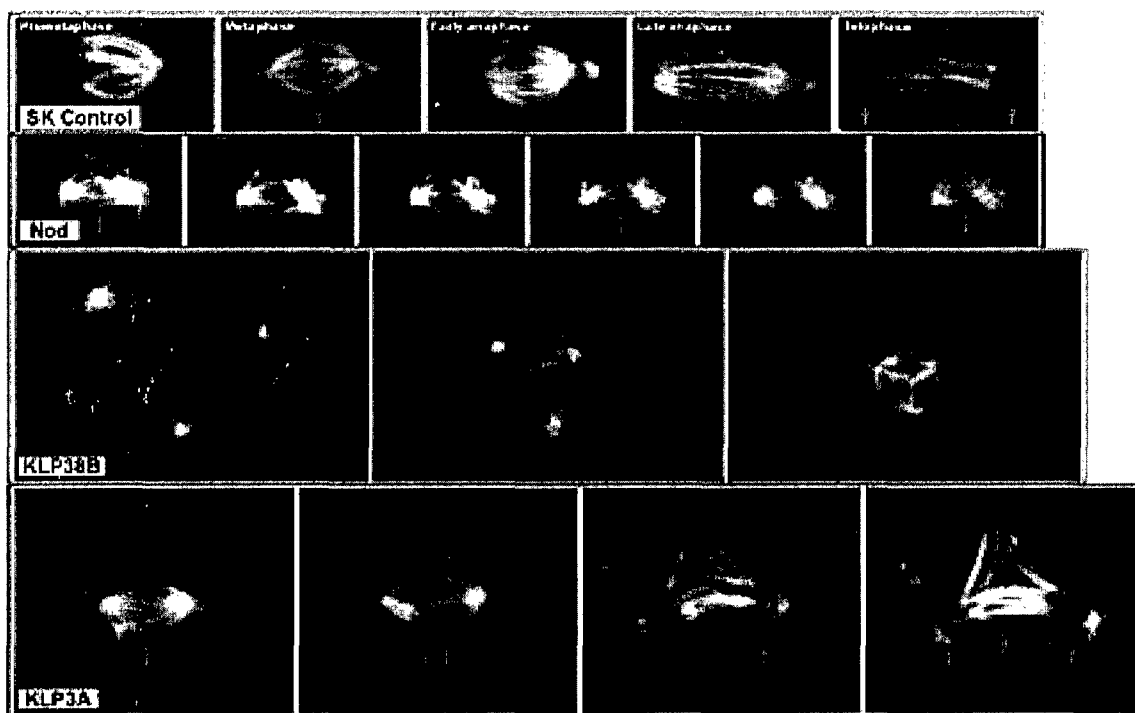


Figure 8. Loss of chromokinesin activity can generate mitotic abnormalities, which presumably result in an increased aneuploidy frequency. Individual frames from movies of live cells are shown. Red arrows indicate the position of chromosome masses.

is apparent by the movement of the poles towards each other. Again, chromosome segregation fails. (The particular cell shown is multipolar, with three poles. Multipolarity is occasionally seen in controls, as well, so this phenotype is not due to KLP38B RNAi, though this treatment might increase the frequency of multipolarity.) Finally, a KLP3A RNAi treated cell constructs a normal bipolar spindle, segregates chromosomes, but has a disrupted central array of spindle microtubules that becomes prominent during anaphase (frame 2). Since this central bundle (midbody) of microtubules is needed for cytokinesis, disruption of this structure by KLP3A RNAi could result in multinucleation.

Loss of Chromokinesin Activity Can Increase the Frequency of Multinucleate Cells:

Multinucleation can arise if cells fail to cytokinesis successfully, resulting in a polyploid condition. The percentage of all interphase cells found to be multinucleate after chromokinesin RNAi was found to nearly double in the case of KLP38B RNAi (Figure 9). This finding is surprising since the preliminary results of live cell analysis (above) suggest that KLP38B RNAi causes spindle collapse and prevents chromosome segregation, which should not result in multinucleate cells. Also surprising is the very minor increase in multinucleation frequency observed after KLP3A RNAi, which, by live cell analysis, appeared to interfere with cytokinesis. However, combined KLP3A and KLP38B RNAi does increase the multinucleation frequency above that obtained with just KLP38B RNAi, suggesting that KLP3A function does have some role in preventing multinucleation. How KLP38B RNAi can generate multinucleate cells will require further live cell analysis.

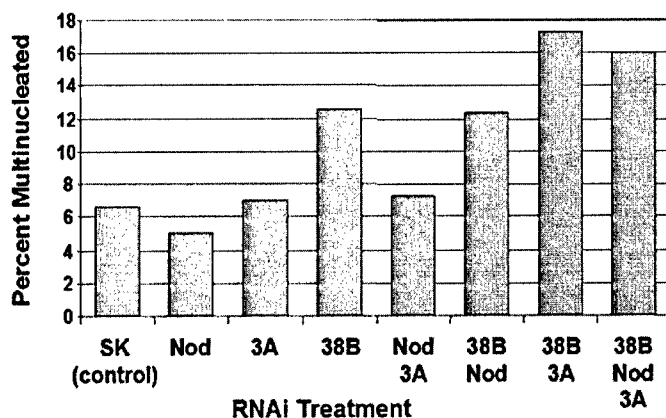


Figure 9. Inhibition of some chromokinesins increases the frequency of multinucleate cells. KLP38B RNAi (individually or when combined with other chromo-kinesin RNAi) appears to be a particularly potent generator of multinucleation.

Loss of Flux Following Chromokinesin Inhibition Might Provide a Mechanistic

Explanation for Increasing Aneuploidy: Flux is a feature of spindle microtubules that has been shown necessary for proper spindle formation and chromosome segregation (13). Spindle microtubules flux as a result of being disassembled at their minus ends (oriented towards poles) while assembling at their plus ends (oriented towards the spindle equator) (Figure 10).

This flow of microtubules towards the poles might be, in part, driven by chromokinesins which would generate a pole-directed force on microtubules while attempting to transport their cargo (chromosomes) to microtubule plus ends (Figure 1B). Loss of chromokinesin activity, therefore, could decrease the rate of flux and disrupt spindle formation and chromosome segregation. We have tested this hypothesis by measuring the flux rate of spindle microtubules in S2 cells (expressing low titers of GFP-tubulin) following RNAi.

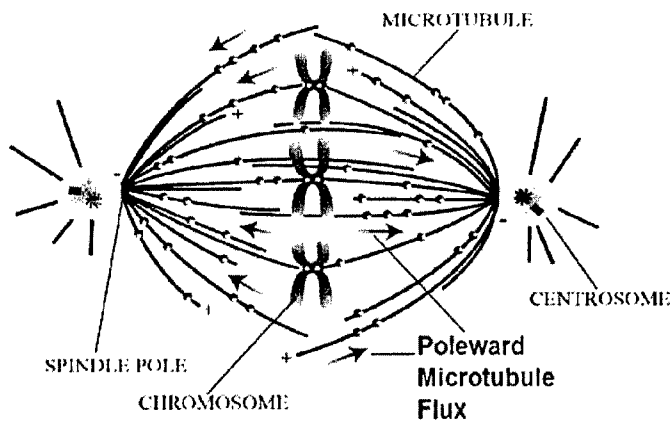


Figure 10. Flux is the movement of tubulin subunits through the microtubules (from plus to minus ends). The flux rate may depend, in part, on the poleward-directed force generated by chromokinesins on microtubules.

To measure flux in RNAi-treated cells, fluorescence speckle microscopy was used to record the movement of fluorescent speckles (GFP-tubulin subunits) along spindle microtubules. Only bipolar spindles were recorded. Flux rates were calculated from the kymographs generated for each speckle movie.

RNAi of any individual chromokinesin was not found to significantly reduce the flux rate relative to the negative control (Figure 11). While only a combination of KLP38B and KLP88A RNAi significantly decreased the flux rate, the treatments of individual Nod RNAi and a combination of KLP38B and KLP31E showed trends to lower flux rates. These preliminary results indicate that a decrease of flux rate might partially account for the increased aneuploidy of chromokinesin-deficient cells, but other mechanisms probably predominate.

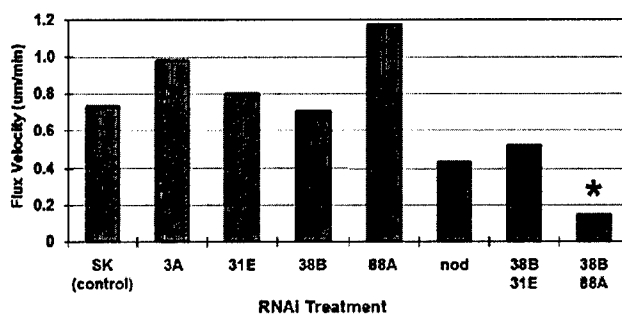


Figure 11. Chromokinesin RNAi generally does not significantly alter the flux rate. Only one combined RNAi treatment was found to decrease the flux rate. Error bars are -SD; asterisk marks significant difference ($P < 0.05$) from control flux rate.

Key Research Accomplishments

- Successfully cloned four of the five putative chromokinesins.
- Generated antibodies to three of the five chromokinesins.
- Demonstrated bona fide chromosome binding by KLP38B.
- Demonstrated that chromokinesin inhibition elevated the mitotic index.
- Demonstrated that chromokinesin inhibition increases the frequency of aneuploidy, and that this aneuploidy is manifested as an increase in chromosome number.
- Demonstrated that chromokinesin inhibition increases the frequency of multinucleation.
- Observed mitosis in live cells following chromokinesin knock-down, so that the specific activities of targeted chromokinesins can be evaluated.
- Demonstrated that chromokinesin inhibition may decrease the flux rate, suggesting that this mechanism could possibly partially explain the loss of proper spindle formation and chromosome segregation after inhibition of chromokinesins.

Reportable Outcomes

The results of these experiments were reported as a poster at the Era of Hope 2005 Department of Defense Breast Cancer Research Program Meeting (poster title: "Inhibition of chromokinesins increases the aneuploid frequency of S2 cells").

These results will also be presented at the national meeting of the American Society of Cell Biologists in San Francisco, CA, December 10-14, 2005.

Currently, we anticipate these data as providing a foundation for two manuscripts for publication: the first will report the phenotypes (eg, increase in aneuploidy, increase in multinucleation, abnormal mitoses) generated by chromokinesin knock-down, while the second will deal in detail with the decrease of the flux rate following chromokinesin RNAi.

We also intend to use these data as the basis for an application for further funding by the DoD Breast Cancer Research Program.

Conclusions

- Disruption of chromokinesin activity can result in aneuploidy.
- Not all chromokinesins have identical effects on aneuploidy.
- Aneuploidy is manifested as an increase in chromatid number. Loss of chromosomes may cause severe inviability.

- Chromokinesin RNAi can also increase the mitotic index and the frequency of multinucleation.
- One process by which loss of chromokinesins may generate aneuploidy is by disrupting flux.

Personnel

Daniel W. Buster and David J. Sharp participated in this project (both as a part-time commitment) and received pay from the project contract.

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